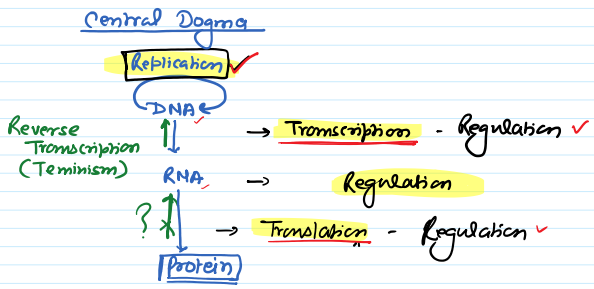
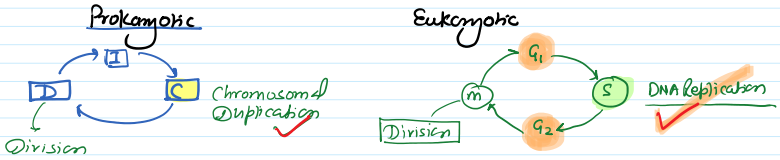
Molecular Biology

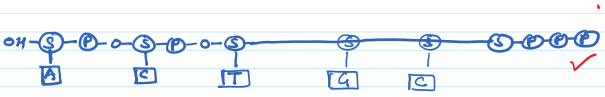


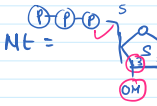
Replication



Replication Charachterstics →

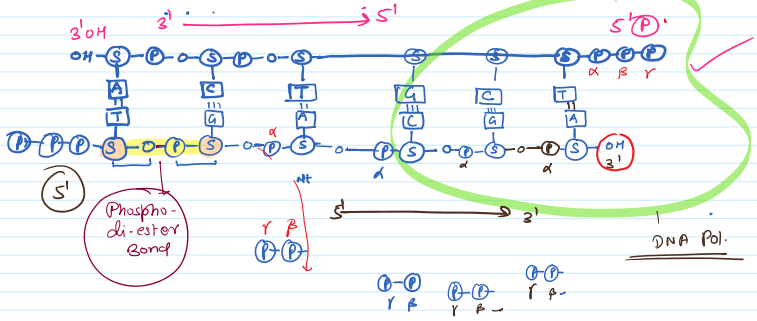
5' → 3' direction





= Adenosine Tri-phosphate (ATP)
Adenine + deoxyribose sugar

deoxyRibose sugar (2nd position - deoxy)



→ OH group - Nucleophillic attack on incoming Nt. and New Nt. is start Polymerizing in 5' → 3' direction

* Energy required for Bond formation obtained from Release of Pyrophosphate (2-p)

(2) Proof reading

→ during Replication Removal of incorrect nt By DNA Pol. itself

direction 3' → 5' Exonuclease activity

PRANSHU (5' → 3')

PRANSHU (5' ← 3')

Exonuclease activity [Proof reading]

(3) DNA Replication is Template Dependent Process

DNA

for DNA Polymarase

↓

DNA Dependent DNA Polymarase

RNA

for Reverse Transcriptase.

- RNA Dependent DNA Polymarase
- Telomarase

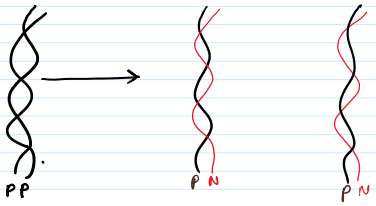
Exception

- Template Independent DNA Polymarase

eg- Terminal deoxy nt. Transferase (Tdt)

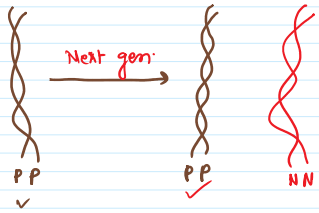
- tnt in B-cell
- used in Blotting tech
- Repair mechanism.

(4) ⇒ mode of Replication is Semiconservative

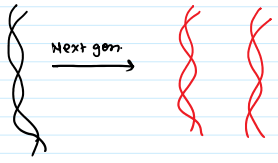


* Before the evedense of Semiconservative mode of Replication There were various Hypothesis-

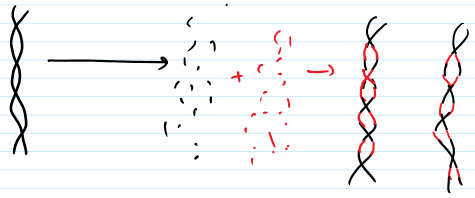
(1) Conservative mode



(2) Non-Conservative -



(3) Dispersive mode of Replication



Meselson and Stahl Experiment -

- Proves that mode of DNA Replication is Semiconservative.

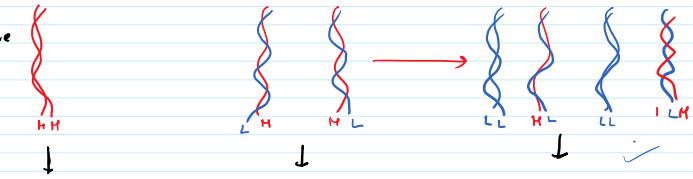
Experiment - E. coli - Cultured on -
N^{15} medium For Several Generation

↓ 1st gen

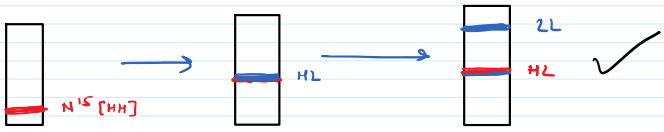
N^{14} medium → 2nd → N^{14} medium

N^{15} medium → 1st gen → N^{14} medium → 2nd gen → N^{14} medium ✓

* if Semiconservative mode

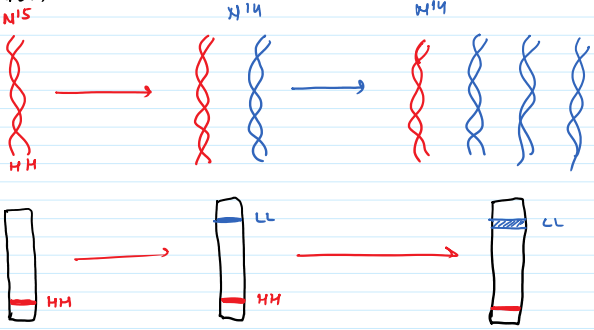


Density Gradient Centrifugation on ($CsCl_2$ solution)

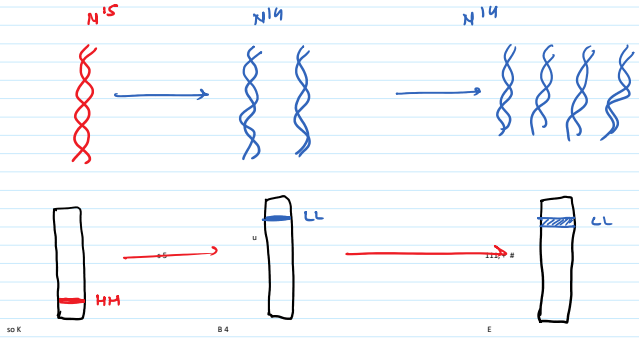


✓

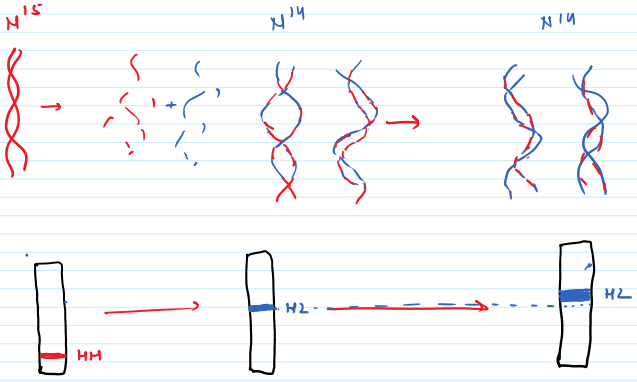
if Replication mode is Conservative



if DNA Replication Non-Conservative



if DNA Replication mode is dispersive →



DNA Replication

① DNA Synthesis → 5' ⟶ 3'

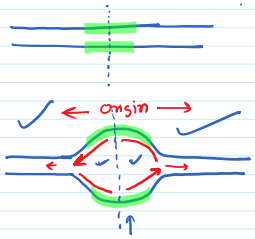
② Proof reading → 3' ⟶ 5' Exonuclease activity

③ DNA Synthesis Template Dependent

④ DNA Replication – Semi conservative

⑤ Replication is Bi directional Process

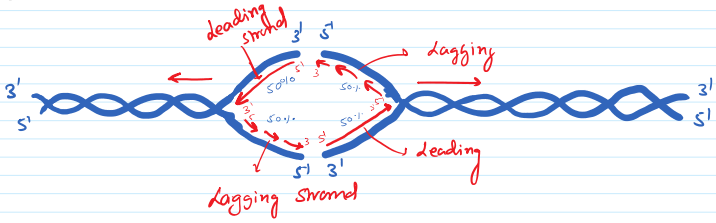
- Replication Start Point = origin of Replication
  - Single → Prokaryote
  - multiple → Eukaryote



Replication Bubble / eye

Replication fork

⑥ Replication is Semidiscontinuous process



(via Okazaki fragments)

⑦ Substrate for DNA Synthesis ⇒

- dNTPs (deoxyribonucleotide)
  - dATP
  - dTTP
  - dGTP
  - dCTP
- NTPs (Ribonucleotide)
  - ↳ Required for Primer Synthesis

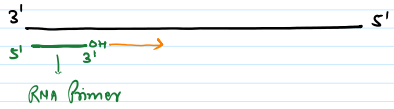
⑧ Primer Required to initiate DNA Replication

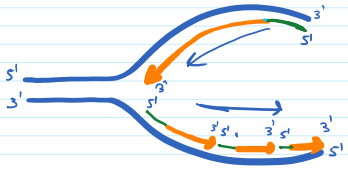
invivo – [RNA. Primer] but PCR (invitro) → DNA Primer

- RNA Primer provide 3'-OH group
  - ↓ Nucleophillic attack on incoming Nt.

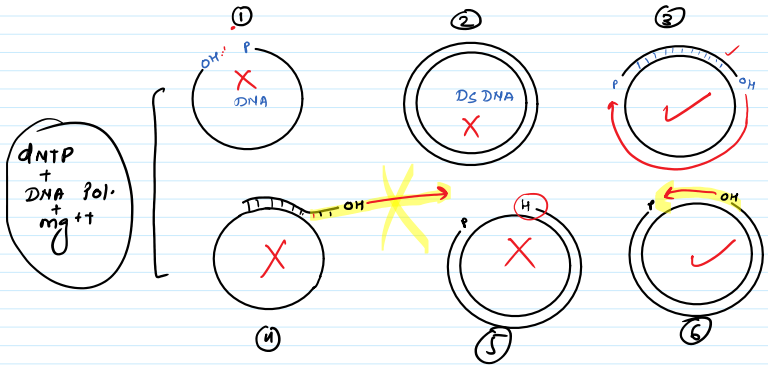
* Template - Primer junction is required for DNA Replication

* DNA Polymerase can not initiate DNA Replication without primer





Que - Invitro DNA Replication Exp.



* Proteins or Enzymes Required in DNA Replication

(1) Initiator protein

→ Recognize origin of Replication Seq.

→ Disrupt H-Bonding at origin site

→ Helps in Recruitment of Helicase

Origin site

Prokaryotic (Single)

E. coli = origin kla

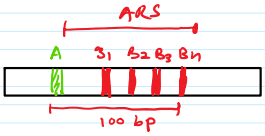
oriC = AT Rich

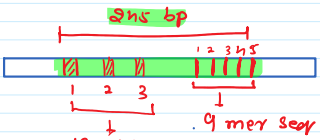
Repetative Seq.

Eukaryotic (multiple)

eg. in Yeast - Autonomous Replicating Seq. [ARS]

AT Rich





13 mer Seq.

9 mer Seq.

Kla Iterons

Repetative Seq.

oriC

ori c or ARS

Recognised by Initiator protein

① Initiator protein

- Prokaryote - DnaA → Recognize ori-c
  DnaC → Helicase loader
  DnaB → Helicase

Eukaryotes - origin recognition complex (ORC) - [1-6] → Recognize ARS

- cdc6
- cdt-1

→ Kia licencing factor → act as Helicase loader

- MCM (2-7) - Kia Helicase

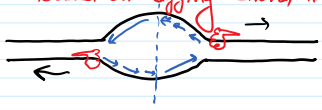
Helicase (2-7)

→ Hexameric protein - (6 subunit)

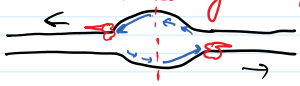
→ Unwinding of DNA by disrupting H-Bond [Using Energy in ATP form]

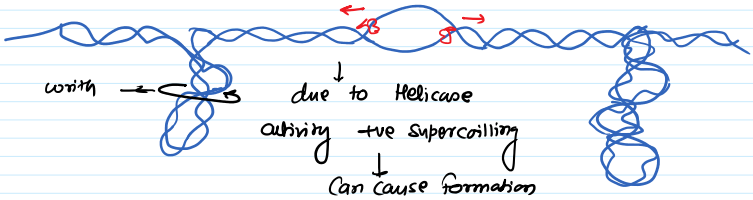
Helicase loading

- Prokaryotes - loaded on lagging strand Template



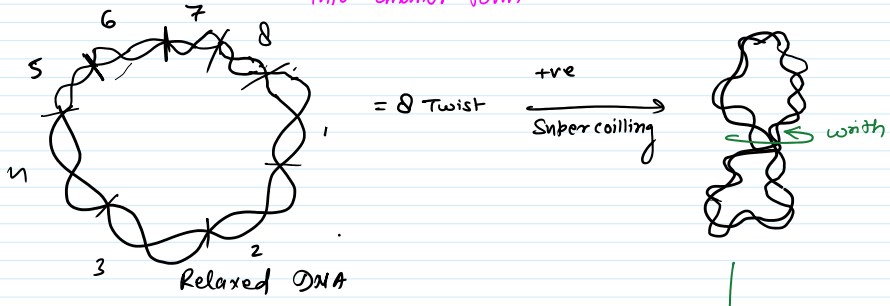
- Eukaryote - loaded on leading strand Template





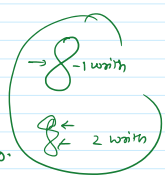
② Topoisomarase

- Change from 1 topological form into another form.



8 Twist → 8 -1 with

8 Twist
+
1 writh
9 = Linking No.



Linking No = Twist + writh
= 8 + 1
L.N. = 9

Topoisomerase brings the change in linking No.

- **Topo**
  - **Topo. I**
    - introduce ss Break on DNA and Change in Linking No. by 1
    - No use of ATP
    - **Topo I**
      - **Topo Ia** → +1
      - **Topo. Ib** → −1
    - Linking No.
  - **Topo. II**
    - introduce ds Break in DNA
    - Reduce linking No. by 2 (−2)
    - Use ATP
    - → induce Negative Supercoiling in DNA

* Inhibitors of Topoisomerase -

- Camptothecin ⊣ Topo. I (Eukaryote)
  [Anticancerous Drug]
- Ciprofloxin
- Norfloxin
  → Bacterial Topo II (Gyrase)
  → Bacteriostatic Antibiotic

Novobiocin ⊣ Topo. II (Gyrase)
Quinolones ⊣ " "